IITRI

Report No. IITRI-L6023-1
(Quarterly Status Report)

LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

Contract No. NASr-22

National Aeronautics and Space
Administration

IIT RESEARCH INSTITUTE

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February 15 to May 15, 1965

National Aeronautics and Space Administration

Contract No. NASr-22
IITRI Project L6023

I. INTRODUCTION

Previous work on this contract has been conducted at IIT Research Institute from February 15, 1961, to February 28, 1965, under IITRI Project C194. Thus, this report follows Report No. IITRI-C194-16.

Studies during this quarter concerned:

(1) Mutation or adaptation of Bacillus cereus and Bacillus subtilis vegetative and spore cells grown in a simulated Martian environment for 7 days

(2) Effects of different freeze-thaw cycles on B. cereus and B. subtilis

(a) 8 hr at -65°C, 16 hr at 25°C

(b) 16 hr at -65°C, 8 hr at 25°C

(c) 20 hr at -65°C, 4 hr at 25°C

(3) Changes in thermal resistance of B. cereus and B. subtilis spores produced in the simulated Martian environment.

The results indicated:

(1) No obvious mutation or adaptation occurred in either B. cereus or B. subtilis cells grown in the simulated Martian environment for 7 days.

(2) Freeze-thaw cycles of different duration affected spore germination, vegetative cell growth, and sporulation of both B. cereus and B. subtilis. Extension of the freeze cycle extended the lag period for spore germination, vegetative cell growth, and sporulation. Notable differences were:

   (a) After 28 days B. cereus total and spore counts from 8- and 16-hr freeze cycles were similar. Results from 20-hr freeze cycle are not complete.

   (b) After 7 days, B. subtilis total and spore counts from the 8-hr freeze cycle were similar, the spore counts from the 16-hr freeze cycle were less than 10% of the total population, and the spore counts from the 20-hr cycle were less than 1%.

   (c) B. subtilis spores will germinate with subsequent vegetative growth with a diurnal 20-hr freeze cycle.

(3) Thermal death time studies on B. subtilis indicated that no change in thermal resistance occurred in spores produced in the simulated Martian environment.

Similar studies with B. cereus were not conclusive because of initially low spore populations.

II. EXPERIMENTAL PROCEDURES

The simulated Martian atmosphere described in Report No. IITRI-C194-5 was used. The methods of inoculating and sampling Mars tubes also have been described in previous reports.

The Bacillus species used were the IITRI strain of B. subtilis and a B. cereus strain isolated from California desert soil. The spore suspensions were prepared in the manner described in Reports No. IITRI-C194-12 and -13. The spore suspensions were heat-shocked for 10 min at 80°C just before use.

Experiments investigating the growth response of B. cereus and B. subtilis to different freeze cycles were conducted in the following manner. Heat-shocked spore suspensions of both organisms were used as inocula.

The environmental conditions were: a diurnal temperature cycle of different durations, and a felsite/limonite soil containing 1% organic medium, 8% moisture, and 15 mm oxygen. At the end of 7 days tubes from the 8-hr freeze cycle were opened and were diluted with 0.1% peptone water. This suspension was used to inoculate other Mars tubes. These tubes were subjected to the same environmental conditions in the same manner as stated above. The procedure was repeated twice. Total and spore counts were performed at once and at 1, 2, 3, 7, 28, and 56 days unless otherwise noted.

Thermal death time studies were conducted with the 7-day 8-hr freeze cycle spore suspensions of B. cereus and B. subtilis by placing 2.5 ml of a spore suspension in 10 x 100-mm cotton-plugged tubes and placing these tubes in an oil bath equilibrated to 90 ± 1°C. Time at 90°C commenced when control tubes reached this temperature. Plate counts were performed in duplicate at once, after reaching test temperature, and after 5, 10, 15, 20, 25, and 30 min at test temperature. Thermal death time curves were plotted as the log number of bacteria surviving heat treatment at a particular time.

In all experiments the recovery medium was trypticase soy agar (BBL). B. cereus was incubated for 24 hr at 35°C and B. subtilis for 48 hr at 35°C.

III. RESULTS AND DISCUSSION

Growth response and spore production data on B. cereus in a simulated Martian environment with different freeze-thaw cycles are presented in Figure 1. There were three groups:

(1) Control Group. No previous contact with the Martian environment; with 2 freeze cycles, 8 and 16 hr.

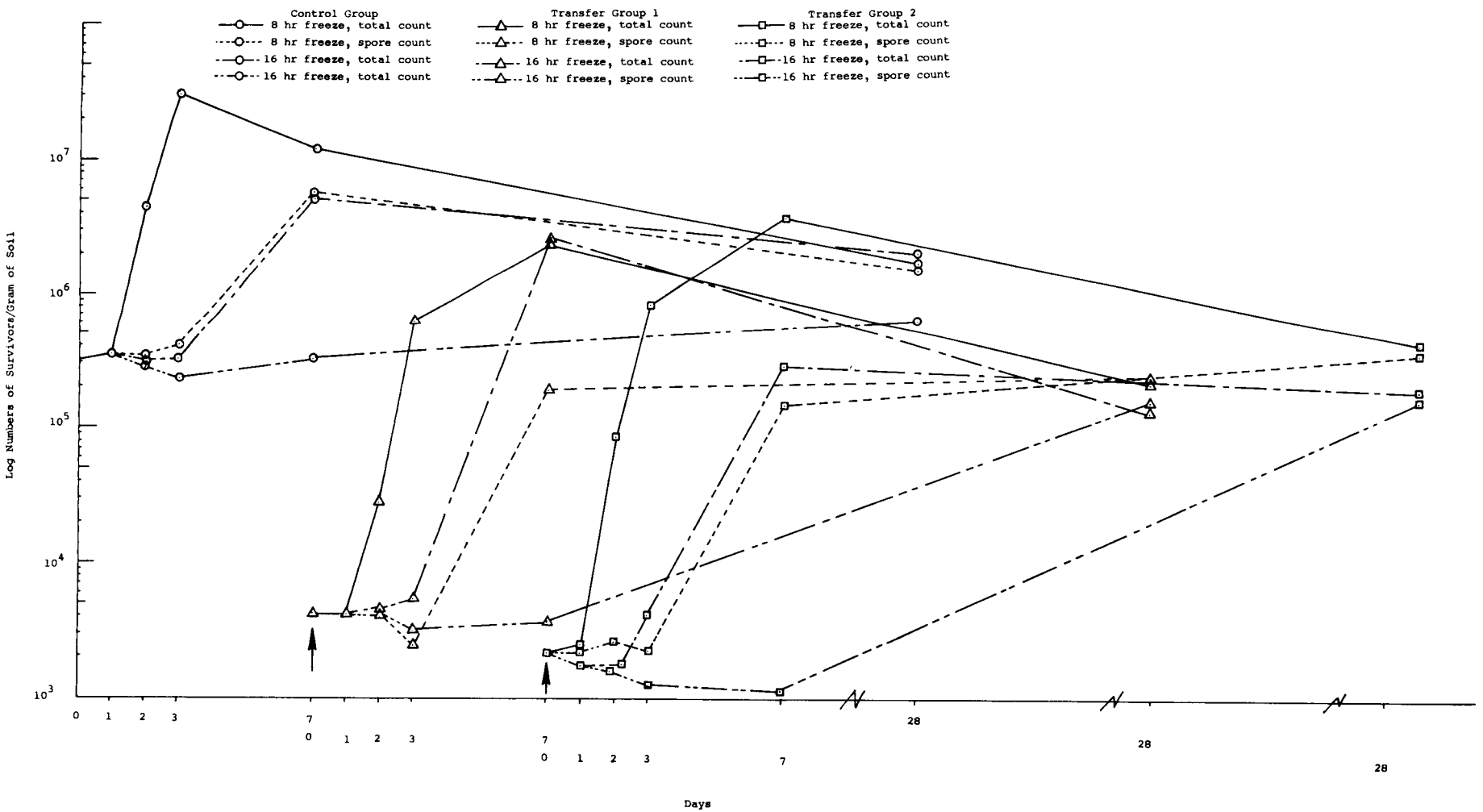
(2) Transfer Group 1. Spores derived from Control Group (8 hr freeze cycle) after 7 days in the simulated Martian environment; with 2 freeze cycles, 8 and 16 hr.

(3) Transfer Group 2. Spores derived from Transfer Group 1 (8-hr freeze cycle) after 7 days in the simulated Martian environment; with 2 freeze cycles, 8 and 16 hr.

Figure 1

THE EFFECT OF DIFFERENT DURATION FREEZE CYCLES ON BACILLUS CEREUS SPORES PRODUCED IN A SIMULATED MARTIAN ENVIRONMENT

Test Conditions: All tubes contained 15 mm O_2, 8% H_2O, and Felsite/Limonite soil with 1% organic medium.



The 8-hr freeze cycle total counts of Transfer Groups 1 and 2 reached maximum numbers after 7 days; this was slower than the Control Group. However, this could be the result of initially lower numbers of bacterial cells present in Transfer Groups 1 and 2. No apparent mutation or adaptation occurred, as evidenced by the similarities in the slopes of comparable growth curves between the groups for both total and spore counts. A possible exception is the 16-hr freeze cycle spore counts from all the groups. The differences in the slopes between these curves could be related to available oxygen. Both the Control and Transfer Group 1 at 7 days had maximum total count populations for the 16-hr freeze cycle -- an order of magnitude, or 90%, higher than Transfer Group 2. If the oxygen concentration for sporulation becomes limiting at this time, the slopes of the Control and Transfer Group 1 would be less than those of Transfer Group 2, which in effect was the case.

Extension of the freeze cycle to 16 hr delayed spore germination and/or cell growth (total count) 48 hr in all groups compared with the 8-hr freeze cycle total counts. Also, extension of the freeze cycle to 16 hr delayed sporulation at least 96 hr in both Transfer Groups compared with the 8-hr freeze cycle spore counts.

The duration of the freeze cycle had no effect on growth in the simulated Martian environment after 28 days. The total and spore counts of both 8- and 16-hr freeze cycles were similar within each group, indicating that surviving cell populations were largely spores capable of germination and subsequent vegetative growth.

Figure 2 presents similar data for B. subtilis except that three different freeze cycle durations were used: 8, 16, and 20 hr. No apparent change occurred in B. subtilis spores produced in the simulated Martian environment, as evidenced by the similarities in the slopes of the various curves compared with the Control Group. The 16-hr freeze cycle delayed spore germination and/or vegetative growth (total count) 24 to 48 hr in all groups. The 20-hr freeze cycle delayed vegetative growth at least 72 hr in all groups. However, after 7 days (168 hr) all freeze cycle total counts for the Control Group and Transfer Groups 1 and 2 were similar.

Sporulation in the different groups was delayed 48 hr with the 16-hr freeze cycle and depressed (extended lag) with the 20-hr freeze cycle. At 7 days the majority of the cells present were spores with the 8-hr freeze cycle, between 70 and 90% spores with the 16-hr freeze cycle, and less than 1% spores with the 20-hr freeze cycle.

Results from thermal death time studies with B. subtilis spores produced in the simulated Martian environment indicated that no change occurred in thermal resistance of spores, as shown in Figure 3.

Results from similar thermal death time studies with B. cereus spores were inconclusive. The initial spore populations were very low with the resultant curves being insignificant.

Figure 2

THE EFFECT OF DIFFERENT DURATION FREEZE CYCLES ON BACILLUS SUBTILIS SPORES PRODUCED IN A SIMULATED MARTIAN ENVIRONMENT

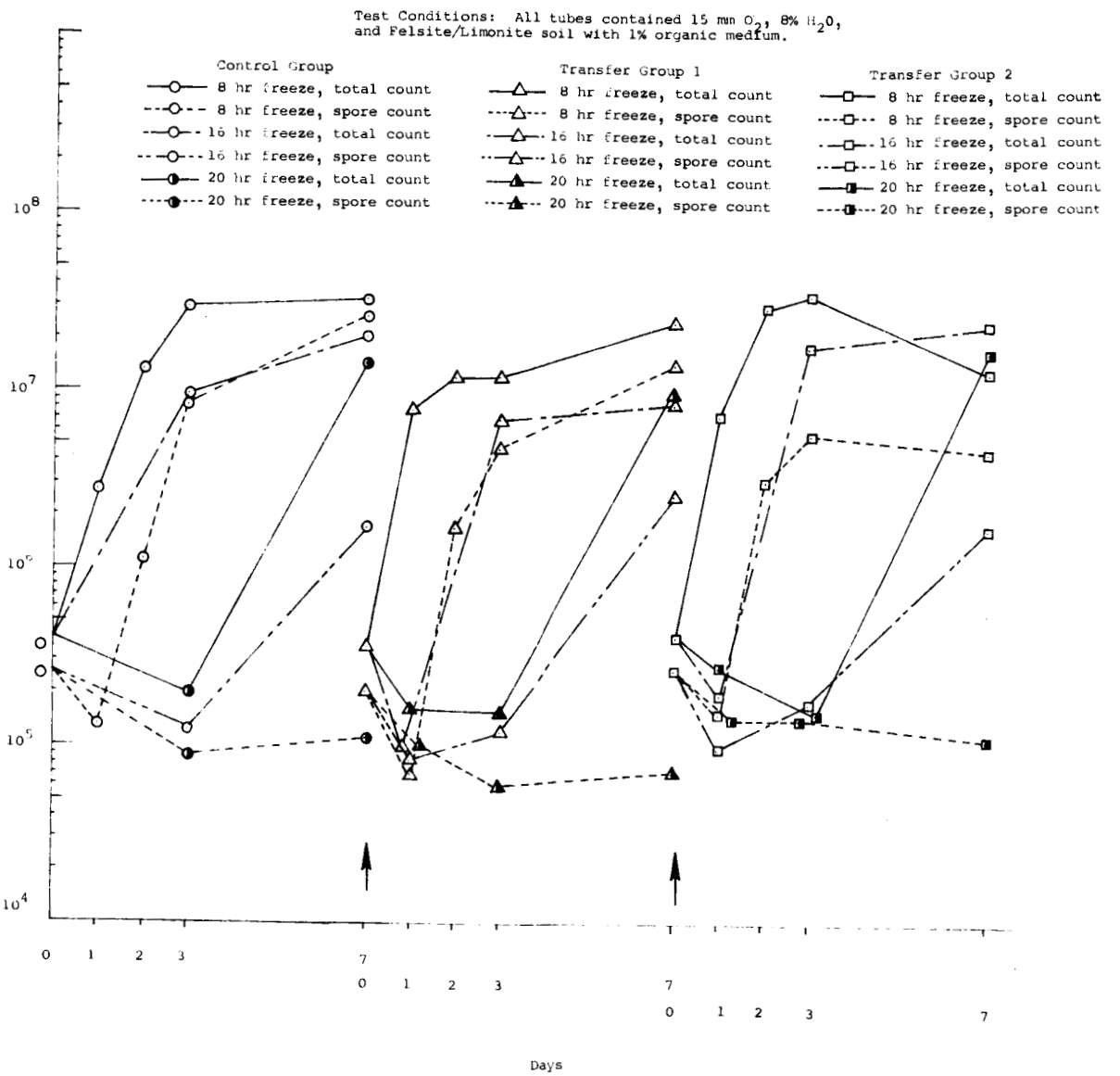
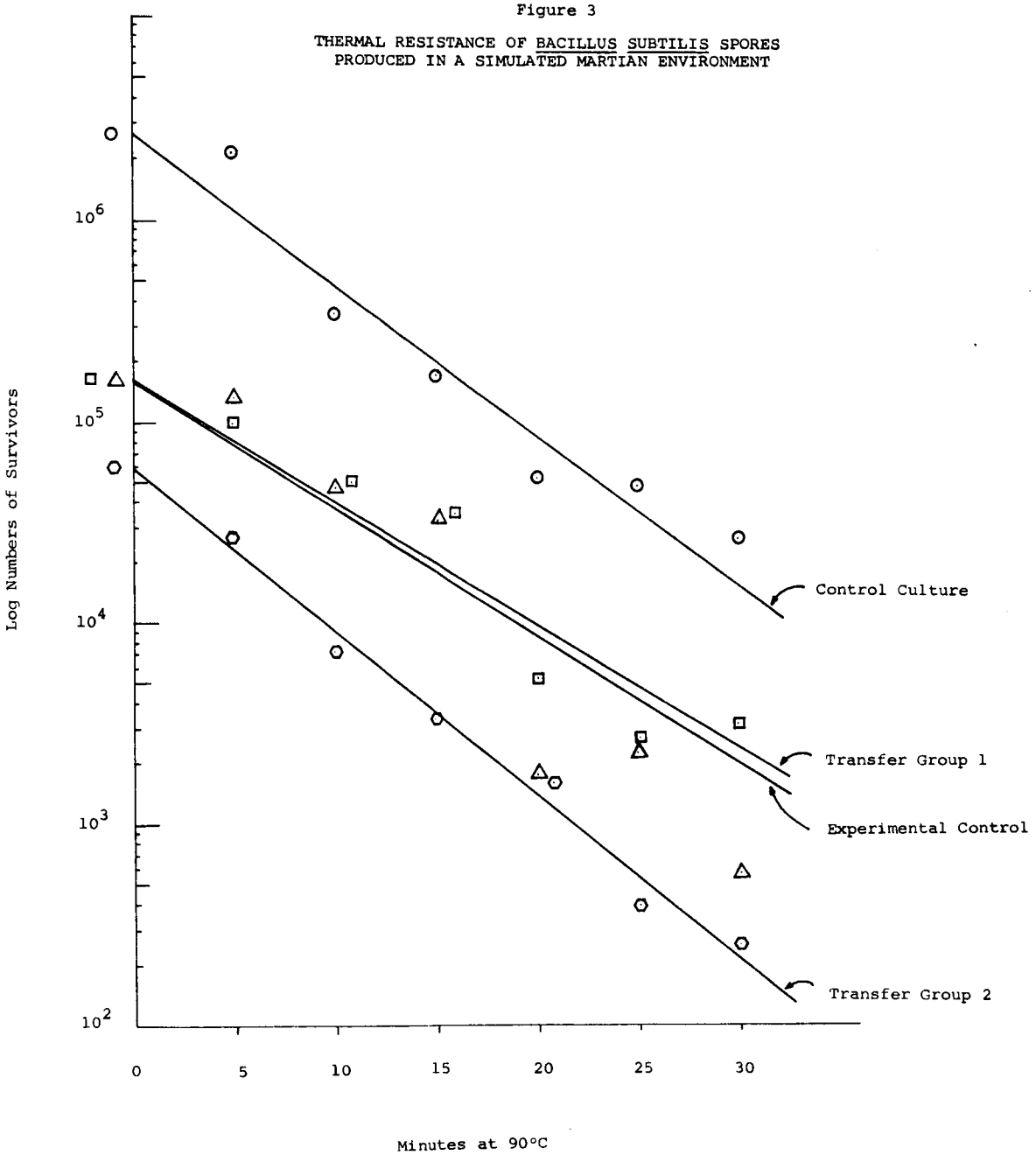


Figure 3

THERMAL RESISTANCE OF BACILLUS SUBTILIS SPORES
PRODUCED IN A SIMULATED MARTIAN ENVIRONMENT



IV. SUMMARY

Growth response studies of B. cereus cells subjected to a modified simulated Martian environment with freeze cycles of different durations indicated that:

(1) No mutation or adaptation occurred within 7 days in spores produced in the environment.

(2) Extension from an 8- to a 16-hr freeze cycle delayed spore germination and/or vegetative growth 48 hr and delayed sporulation more than 96 hr.

(3) By 28 days total and spore counts from 8- and 16-hr freeze cycles were similar.

Studies with B. subtilis cells indicated that:

(1) No mutation or adaptation occurred within 7 days in spores produced in the environment.

(2) Extension from an 8- to a 16- or a 20-hr freeze cycle delayed spore germination and/or vegetative growth at least 48 and 72 hr, respectively. Sporulation was delayed 48 hr when the freeze cycle was extended to 16 hr and was depressed when the freeze cycle was extended to 20 hr.

(3) A quantitative difference in numbers of spores resulted from extension of freeze cycles. With an 8-hr freeze cycle more than 90% of the cells surviving after 7 days were spores compared with 70 to 90% and less than 1% with the 16- and 20-hr freeze cycles, respectively.

V. PERSONNEL AND RECORDS

Experiments were planned with the counsel of Dr. E. J. Hawrylewicz, and technical assistance was given by Miss Charlene Berger and Mr. John Collum.

Experimental data are recorded in Logbooks C15491 and C15783.

Respectfully submitted,

IIT RESEARCH INSTITUTE

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